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Evaluation of thiol/disulfide homeostasis in patients with acne vulgaris

Thiol/disulfide homeostasis and acne vulgaris

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Abstract

Aim: Acne vulgaris (AV) is a chronic, multifactorial, inflammatory disease of the pilosebaceous unit. In this study, we compare the dynamic thiol/disulfide homeostasis (TDH) of patients with AV with that of control subjects. Material and Method: In this study, the dynamic TDH in patients with AV was measured and compared with that of the control subjects using the method following Erel and Neselioglu. Results: The patient group comprised 38 female and 36 male patients, and the control group comprised 29 female and 31 male subjects. The total thiol levels were significantly lower in patients with AV than in the control subjects (p=0.044), and the disulfide/total thiol ratio was significantly lower in patients with AV (p=0.034). Plasma native thiol, total thiol, and disulfide levels did not differ significantly between the subgroups (p>0.05), and the disulfide/native thiol ratio, disulfide/total thiol ratio, and native thiol/total thiol ratio did not differ significantly among the patients with mild, moderate, and severe disease (p>0.05). Discussion: To our knowledge, this is the first study to evaluate TDH in patients with AV. Plasma thiol levels, an important component of the antioxidant system, are found to be significantly lower in AV.

Keywords

Acne Vulgaris; Oxidative Stress; Thiol; Disulfide

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Introduction

Acne vulgaris (AV) is a chronic, multifactorial, inflammatory disease of the pilosebaceous unit [1] and is considered to be the most common dermatological disease, affecting 80% of people aged 11–30 years [2]. There are multiple factors that play a role in AV pathogenesis, with the follicular hyperkeratinization, sebaceous hyperplasia and colonization of *Propionibacterium acnes* being the main causes of acne formation, and inflammation and immune reactions playing key roles in disease pathogenesis [3]. In addition, diet, nutrition, genetic factors and oxidative stress play a role in the pathogenesis of acne [4], and there is substantial experimental evidence that supports the initiating role of oxidative stress in the onset of acne [5].

Thiols are sulfur-containing organic compounds that form complexes with amino acids and proteins and are essential for biological systems [6]. Thiols are key antioxidants that play a crucial role in the disposal of reactive oxygen species through enzymatic and non-enzymatic pathways. The plasma thiol pool consists of low molecular weight thiols – such as cysteine, homocysteine, glutathione and albumin – and, in particular, protein-thiol compounds. Thiols undergo oxidation reactions to form disulfide bonds with oxidant molecules, and dynamic thiol/disulfide homeostasis (TDH) is essential for detoxification, for the regulation of signaling pathways, and for apoptosis and enzymatic reactions [7,8]. Studies showed that dysregulated TDH is associated with the pathogenesis of various diseases, including diabetes, cardiovascular diseases, malignant tumors, Parkinson's disease, Alzheimer's disease and multiple sclerosis [9-12].

In this study, we compare the dynamic TDH of patients with AV with that of control subjects. To our knowledge, this is the first study to date to evaluate TDH in patients with AV.

Material and Method

Included in the study sample were 74 patients who were admitted to the dermatology clinic and who received a clinical AV diagnosis, and the control group comprised 60 age- and gender-matched healthy subjects. Ethical approval was obtained before this study, and all participants involved provided written informed consent. The criteria for exclusion were as follows: Presence of chronic inflammatory disease,use of alcohol or tobacco, and being in a gestation or lactation period. The Global Acne Evaluation system was used to classify the disease severity of the patients as mild, moderate or severe AV (Table 1).

Fasting venous blood samples were placed in 10 mL vacuum tubes (Vacutainer BD) and stored at -80°C until the point of analysis. The automated method described initially by Erel and Neselioglu (Cobas c501, Roche Diagnostics, Indianapolis, Indiana, USA) was used for the measurement of serum disulfide/thiol homeostasis.

Statistical analysis

Analyses were performed using the "Statistical Package for Social Sciences for Windows" (SPSS v19) software package. The relevance values of the normal distribution were tested with a Kolmogorov-Smirnov test. Parametric tests were expressed as mean±standard deviation, while non-parametric tests were expressed as median (min-max). A Student's t-test was applied for parametric tests, and a Mann-Whitney U-test was used for non-parametric tests of both the patient and control groups. Differences between the subgroups of the global acne grading system were assessed with a Kruskal-Wallis test for non-parametric variables, and by an ANOVA for parametric variables. A *p*-value of <0.05 was considered statistically significant.

Results

The patient group comprised 38 female and 36 male patients and the control group comprised 29 female and 31 male subjects. The mean age was 18.54±3.40 years in the patient group, and 17.68±3.37 years in the control group. The patient and control groups were similar concerning age and gender (p>0.05) (Table 2).

Native thiol levels did not differ significantly between patients with AV (417.63±42.42 µmol/L) and the control subjects (430.22±54.72 µmol/L) (p=0.136). Total thiol levels were significantly lower in the patients with AV (455.84±47.47 µmol/L) than in the control subjects (474.32±57.99 µmol/L) (p=0.044). Plasma disulfide levels, on the other hand, did not differ significantly between patients with AV (19.44±7.20 µmol/L) and the control subjects (21.57±5.52 µmol/L) (p=0.069). Similarly, the disulfide/native thiol ratio and native thiol/total thiol ratio did not differ significantly between the patients with AV and the control subjects (p=0.065 and p=0.069, respectively). The disulfide/total thiol ratio was significantly lower in patients with AV (p=0.034) (Table 2).

Patients were classified into three subgroups based on the Global Acne Evaluation system of mild (n=25), moderate (n=24) and severe (n=25). Plasma native thiol, total thiol and disulfide levels did not differ significantly between the subgroups (p>0.05), and the disulfide/native thiol ratio, disulfide/total thiol ratio, and native thiol/total thiol ratio did not differ significantly among the patients with mild, moderate and severe disease (p>0.05) (Table 3).

Discussion

The skin is the largest organ in the body and is exposed to the highest level of oxidative stress. Compared to the dermis, the epidermis has a higher concentration of antioxidants, and this concentration gradient increases in the deeper layers of the stratum corneum. Oxidative stress is known to affect multiple cutaneous diseases [13].

Propionibacterium acnes plays an initiating role in AV by producing low molecular weight chemotactic factors that cause a neutrophil accumulation in acne comedones. Following phagocytosis, neutrophils secrete various inflammatory substances (such as lysosomal enzymes), which in turn damage the follicular epithelium. Moreover, neutrophils, which are located in regions of inflammation synthesize hydroxyl radicals, superoxide anions and other strong reactive oxygen species, resulting in tissue damage. The reactive oxygen species that are synthesized in neutrophils are closely associated with the pathogenesis of various inflammatory skin diseases [3,14,15]. Basak et al. determined a reduction in the antioxidant levels of leukocytes in patients with AV (e.g. superoxide dismutase and glutathione peroxidase), and an increase in serum lipid peroxidation [16]. El-Akawi et al. found that antioxidants, such as vitamin A and vita-

Table 1. Global acne grading system

Location	Factor
Forehead	2
Right cheek	2
Left cheek	2
Nose	1
Chin	1
Chest and upper back	3

Each type of lesion is given a value based on severity: no lesions = 0, comedones = 1, papules = 2, pustules = 3 and nodules = 4. The score for each area (Local score) is calculated using the formula: local score = factor × grade (0–4). The global score is the sum of the local scores, and acne severity was graded using the global score. A score of 1–18 is considered mild; 19–30, moderate; 31–38, severe; and >39, very severe.

Table 2. Baseline characteristics and plasma thiol-disulfide levels of acne vulgaris and control group

Parameter	Acne vulgaris N=74	Control N=60	P
Age	18.54±3.40	17.68±3.37	0.14
Sex (Male/Female)	36/38	31/29	0.72*
Native thiol (μmol/L)	417.63±42.42	430.22±54.72	0.136
Total thiol (μmol/L)	455.84±47.47	474.32±57.99	0.044#
Disulphide (μmol/L)	19.44±7.20	21.57±5.52	0.069
Disulphide/native thiol (%)	4.58±1.74	5.18±1.94	0.065
Disulphide/total thiol (%)	4.15±1.45	4.69±1.43	0.034*
Native thiol/total thiol (%)	91.68±2.90	90.71±3.20	0.069

#Chi-squared
*p<0.05

Table 3. Plasma thiol-disulfide levels according to the global acne grading system

Parameter	Mild acne N=25	Moderate acne N=24	Severe acne N=25	P
Native thiol (μmol/L)	406.37±43.93	414.72±41.91	31.68±38.90	0.098
Total thiol (μmol/L)	449.67±55.14	449.31±44.65	468.28±40.72	0.277
Disulphide (μmol/L)	21.65±9.12	17.29±5.12	19.28±6.23	0.105
Disulphide/native thiol (%)	5.27±2.02	4.19±1.27	4.27±1.70	0.053
Disulphide/total thiol (%)	4.70±1.65	3.84±1.08	3.89±1.42	0.062
Native thiol/total thiol (%)	90.58±3.31	92.30±2.17	92.20±2.85	0.062

min E, are reduced in patients with AV [17]. Antioxidants, such as sodium ascorbyl phosphate (vitamin C precursor), topical/oral zinc and nicotinamide, are used to treat acne lesions [18]. Dynamic TDH has been explored for different disease states. Erel et al. found higher disulfide levels in patients with inflammatory diseases, such as obesity, pneumonia and diabetes [19]. Dogru et al. determined a marked reduction in thiol levels in patients with ankylosing spondylitis and showed that thiol levels are reduced even more during the activation period of the disease. Based on these results, the authors concluded that antioxidant systems are affected by inflammatory diseases, especially during the active periods [7]. In another study, Kundi et al. identified a correlation between disease severity and thiol levels in patients with coronary atherosclerosis; the mortality rate was higher in patients with lower thiol levels [20]. Ates et al. determined a positive correlation between blood glucose levels and disulfide levels in prediabetic patients [21]. More recently, Kaplan et al. evaluated dynamic TDH in patients with celiac disease, and revealed that patients with celiac disease have lower total and native thiol levels than control subjects, but higher disulfide levels, disulfide/total thiol ratios and disulfide/native thiol ratios [22]. Elmas et al. looked for correlations between dynamic TDH and inflammatory cardiovascular markers in obese children and determined a positive correlation between oxidant parameters, body-mass index (BMI) and inflammation markers [23]. Demirseren et al. evaluated dynamic TDH in patients with basal cell carcinoma, and found a significant reduction in disulfide levels and increased native thiol levels. In light of these findings, the authors concluded that TDH may be dysregulated in the proliferative phase of the disease [24].

In this study, native thiol levels did not differ significantly between patients with AV and the control subjects, while total thiol levels were found to be significantly lower in patients with AV. Plasma disulfide levels did not differ significantly between the patients with AV and the control subjects. While disulfide/native thiol and native thiol/total thiol ratios did not differ significantly between the groups, the disulfide/total thiol ratio was significantly lower in patients with AV. Similar to our study, Dogru et al. found significantly lower total thiol levels and disulfide/total thiol ratios in patients with ankylosing spondylitis. However, native thiol levels did not differ significantly between the patients and control subjects [7]. Aynali et al. identified no significant difference in thiol/disulfide levels between antinuclear antibody-positive and antinuclear antibody-negative serum samples [25], while in the present study, we identified no significant differences in the plasma native thiol levels, total thiol levels, disulfide levels, disulfide/native thiol ratios, disulfide/total thiol ratios or native thiol/total thiol ratios. On the other hand, Dogru et al. identified a negative correlation between disease severity and native thiol levels and total thiol levels in patients with ankylosing spondylitis [7], which may be related to the higher intensity of systemic inflammation in ankylosing spondylitis when compared to AV.

Our results indicate that plasma thiol levels, an important component of the antioxidant system, are significantly lower in AV, which suggests that antioxidant systems are affected by inflammatory diseases. Thus, considering the lack of significant differences in thiol levels among the disease subgroups, it is likely that this dysregulation is independent of disease severity. In our study, lower disulfide levels in patients with AV resulted in lower disulfide/native thiol and disulfide/total thiol ratios in the patients than in the control subjects. The oxidative stress associated with AV may result from a mechanism that is independent of disulfide levels. Given the multifactorial nature of AV, there are several etiological factors that may produce simultaneous effects. Reduced antioxidant response as a result of the dysregulation of oxidant-antioxidant equilibrium in AV is another possibility.

Inflammation in AV has been attributed to both elevated sebum secretions and the colonization of *Propionibacterium acnes*. However, these causes fail to explain the etiopathogenesis of this multifactorial disease, and current studies tend to focus more on inflammation-oxidative stress and oxidative stress-acne relationships. To our knowledge, there are not any studies

that have evaluated dynamic TDH in patients with AV. Accordingly, prospective studies, including larger patient populations, may help provide a better understanding of the role of TDH in AV pathogenesis.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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